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"31st Ann. Proc. Electron Microscopy Soc. Amer."
New Orleans, La., 1973. C. J. Arceneaux (ed.).



# PROLONGED FIXATION STUDIES FOR SPACEFLIGHT

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Observation of subtle or early signs of change in spaceflight induced alterations on living systems require precise methods of sampling. In-flight analysis would be preferable but constraints of time, equipment, personnel and cost dictate the necessity for prolonged storage before retrieval. Because of this, various tissues have been stored in fixatives and combinations of fixatives and observed at various time intervals. High pressure and the effect of buffer alone have also been tried.

Of the various tissues embedded, muscle, cartilage and liver, liver has been the most extensively studied because it contains large numbers of organelles common to all tissues (Fig. 1). The fixatives tested were 2% paraformaldehyde, 6% glutaraldehyde, 3% glutaraldehyde with 1% paraformaldehyde, and each of the previous three plus 0.05% dinitrodifluorobenzene (DFF). These were all buffered with s-collidine and contained 0.25% 0.1 M $CaCl_2$. Each of the six basic solutions was made 1%, 5% and 10% sucrose, resulting in 18 different fixatives. Tissues were removed and observed at various periods up to 1 year.

The best fixative was the combination of 3% glutaraldehyde, 1% paraformaldehyde and 0.05% DFF in 5% sucrose followed by 1% osmium tetroxide prior to dehydration and embedding (Fig. 2). Combinations of fixatives appeared to offer the best answer since no one fixative completely fixes an entire tissue.

During storage several changes took place in the less favorable fixing solutions. There was generally a decrease in definition of the structures of the tissue especially in the cristae of the mitochondria and the rough and smooth endoplasmic reticulum. The tissue was often expanded in the 1% sucrose and shrunken in the 10% sucrose solutions. Some of the glycogen granules appeared to leach out or change and membrane pools formed and enlarged (Fig. 3). The high pressure preservation, 10 kilobars, for three days preserved the membranes but not the microtubules. Our hope was that the high pressure would inactivate the enzymes thereby preserving the tissue, but preservation was not satisfactory. Control liver kept in buffer for three days before fixation remained quite well preserved. The osmotic pressure of the buffer was carefully adjusted to be isotonic. The ribosomes were retained and easily seen in the nucleus and cytoplasm (Fig. 4) perhaps because general cytoplasmic leaching caused them to stand out.

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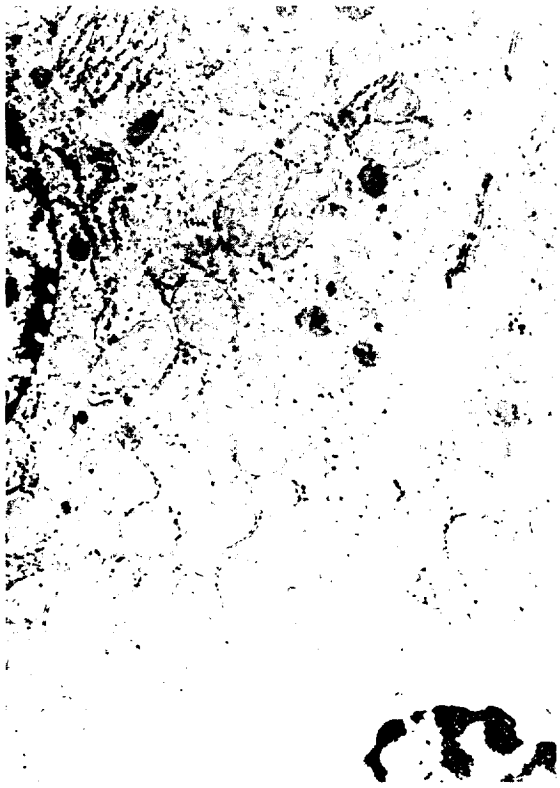

Fig. 1 Rat Liver, one-day control in gluteraldehyde, paraformaldehyde, and DFF. 9650X

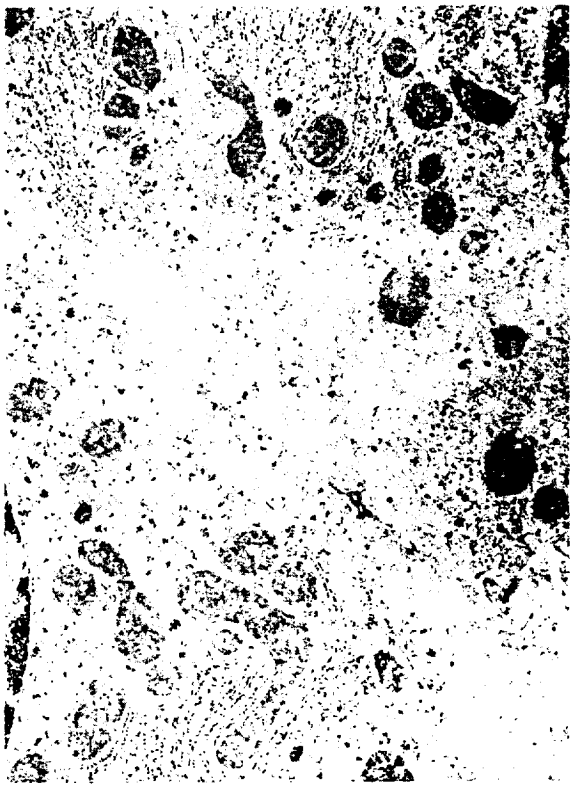

Fig. 2 Rat Liver, three months at room temperature in gluteraldehyde, paraformaldehyde, and DFF. 9100X

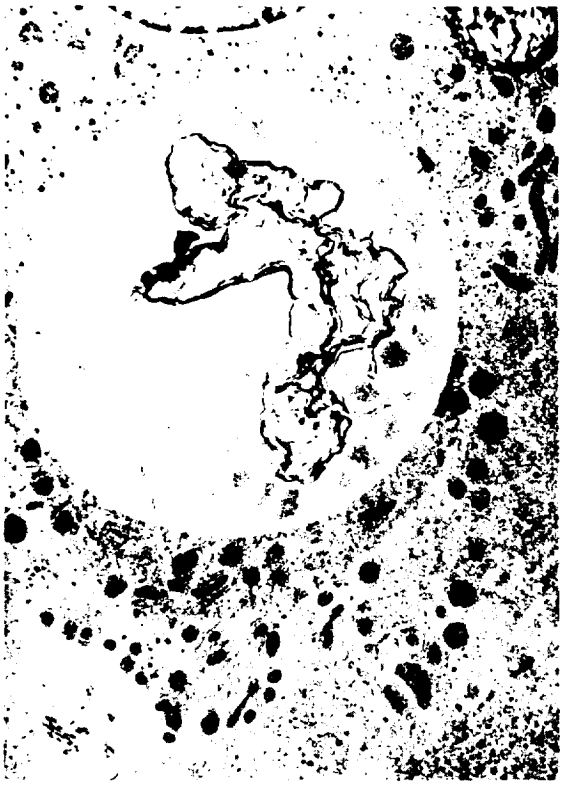

Fig. 3 Rat Liver, three months at room temperature in gluteraldehyde. 6350X

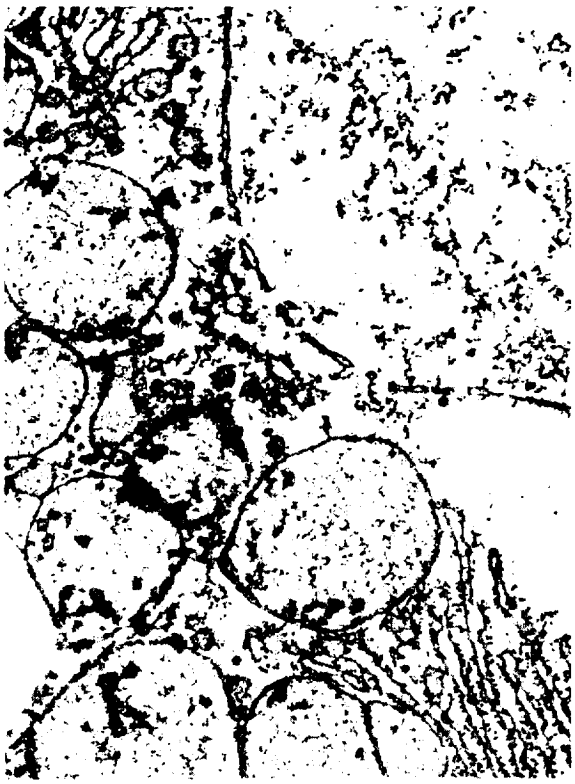

Fig. 4 Rat Liver, non-pressurized control three days in buffer. 26,400X